

1. Scientific Abstract

This is a Phase 1 clinical trial proposal to examine the potential toxicity and efficacy of irradiated autologous tumor cells adenovirally-transduced with human GM-CSF gene. Bam H1 and Eco R1 restriction sites are added to human GM-CSF cDNA (pcD-hGM, ATCC #57594) by PCR using 5' primer (CGC-GGATCCATGTGGCTGCAGAGCCTG) and 3' primer (CCGGAATTCTCACTCCTGGACTGGCTC). Resulting cDNA will be inserted into the polycloning linker sites of the plasmid pCA14 (Microbix Biosystems Inc., Toronto, Canada). The plasmid pCA14 is one of a series of bacterial plasmids comprising circular form of the adenovirus type 5 genome developed by Graham *et al.* [1, 2]. This plasmid lacks the early region 1 (E1) required for virus replication and contains the human cytomegalovirus immediate early promoter/enhancer (bp -299 to 72), a polycloning linker containing unique restriction sites, and the SV40 polyadenylation signals. Recombinant adenovirus will be produced by co-transfecting 293 cells (human embryonic kidney cells) with pCA14 harboring hGM-CSF cDNA and pJM17 by the calcium phosphate method (The plasmid pJM17 is noninfectious in single transfections of 293 cells, since it contains an insertion of a pBR322 derivative at bp1339 in type 5 adenovirus sequence which makes the resulting viral genome too large to package). Replication incompetent adenoviruses harboring hGM-CSF cDNA produced will be denoted as Adv/hGM-CSF. Primary cancer cells established from malignant cancer (head and neck tumor, breast cancer and soft tissue sarcoma) tissues surgically obtained will be infected with Adv/hGM-CSF and irradiated to eliminate the tumorigenicity. The level of GM-CSF production by irradiated GM-CSF gene-transduced tumor cells will be assayed and adjusted to about 20-40 ng/10⁵/24 hr by addition of irradiated autologous tumor cells. These irradiated GM-CSF-producing autologous tumor cells will be used as vaccine. Three different dose levels (from 3 x 10⁷ to 1.2 x 10⁸ cells/individual patient) will be used to examine the toxicity of vaccine and the response of patients in the entire period of 84 days. Each injection will be 1 x 10⁷ irradiated GM-CSF cancer cells suspended in 0.5 cc. One half of the injection will be given intradermally and the other half will be administered subcutaneously. A total of 15 patients will be included in the phase 1 clinical trial program. The entire project will be continued a period of one year.